EFFECT OF SANITIZER TREATMENTS ON *SALMONELLA ENTERICA* SEROTYPE POONA ON THE SURFACE OF CANTALOUPE AND CELL TRANSFER TO THE INTERNAL TISSUE DURING CUTTING PRACTICES

A Thesis

by

SASI VADLAMUDI

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

December 2004

Major Subject: Food Science and Technology

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ABSTRACT

Effect of Sanitizer Treatments on *Salmonella enterica* Serotype Poona on the Surface of Cantaloupe and Cell Transfer to the Internal Tissue during Cutting Practices. (December 2004)

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In recent years, due to the increasing number of produce-related outbreaks, greater attention has been given to interventions that remove human pathogens on fresh produce. Survival of *Salmonella* Poona on the surface and stem scar portions of inoculated cantaloupe, effect of chlorine or lactic acid or ozone on the survival of bacteria, transfer of pathogen from the rind to the fresh-cut tissue during cutting and growth and survival of *Salmonella* Poona on the fresh-cut tissues during 15 days of refrigerated storage were investigated. Preliminary studies were conducted to confirm that the rifampicin-resistant strain used in the study was indistinguishable from the parent strain of *Salmonella* Poona. Growth curve, heat tolerance and lactic acid resistance studies were performed, all of which showed no differences in behavior between the organisms. Cantaloupes were immersed in an inoculum containing rifampicicn-resistant strain of *Salmonella* Poona (10^7 CFU/ml) for 3 min and then dried for 12 h. The inoculated melons were washed with chlorine (200 ppm) for 3 min or lactic acid (2%) for 2 min or ozone (30 ppm) for 5 min and fresh cut tissues were prepared by peeling the rind and cutting into pieces or cutting the melon and removing the rind.

Results obtained indicate that the levels of *Salmonella* Poona recovered were higher when the sample was obtained from the scar portion than the surface. Surface treatment with tap water or chlorine did not reduce *Salmonella* Poona. However treatment with lactic acid or ozone reduced the levels by 2.5 and 2.3 $\log_{10}$ CFU/cm^2 respectively on the surface. Fresh-cut tissue prepared from melons sanitized with lactic acid resulted in less transfer during cutting and reached below detectable limits after 9 days of refrigerated storage. Cutting melons after peeling the rind was found effective in reducing the transfer of *Salmonella* Poona into the tissue in comparison with cutting of melons and removing the rind later. Thus these data suggest that treatment with lactic acid and ozone may be effective in reducing *Salmonella* from the surface of the cantaloupe where as lactic acid was effective in reducing the transfer from the surface to the flesh.

DEDICATION

To my parents, in-laws and my husband Raj Kumar whose support and blessings gave me the ability to successfully complete the project.

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INTRODUCTION

Food, shelter and clothing are the essential items which human beings need for survival. However, man can live without clothing though he faces certain discomforts or inconveniences in life. Similarly he can pull on in life without shelter in spite of the fact that he has to pass through innumerable physical suffering and agonies. But "Food" is an indispensable commodity without which man cannot survive beyond a few days or at best for not more than a month or two.

Human beings, in the past, had to depend on the food which was available locally. The consumption of fruits and vegetables has increased over the past decade due to the change in dietary habits, health trends and increased availability all round the year. The consumption of fruits and vegetables increased by 17% from 1970 to 2002 (ERS 2004). The number of documented outbreaks of human infections associated with consumption of raw fruits and vegetables has increased in recent years due to pathogenic organisms such as *Salmonella*, *Shigella* and *Escherichia coli* O157:H7 (NACMCF 1999). Since 1990, many different types of produce have been linked with outbreaks of food-borne illness in the United States. Per capita consumption of fruits grew by 8% to 129 kg at an average annual rate of 0.33%. Per capita consumption of melons increased by 6% out of which cantaloupes contribute around 2% (ERS 2004). Cantaloupes, in particular, have been associated with several multi-state *Salmonella* outbreaks during the last decade.

This thesis follows the style of the Journal of Food Science.

Salmonella is a facultative anaerobic gram-negative bacterium that does not require strict conditions for its growth. It is capable of proliferating and surviving in diverse ecosystems including in food production and processing facilities. Environmental stresses such as osmotic shock, oxidative stress, heat shock, low pH and nutrient starvation are the contributing factors in the capability of *Salmonella* to proliferate in diverse ecosystems (Kwon and Rickie 1998). Infection in humans is characterized by gastroenteritis which manifests itself as diarrhea, vomiting, fever, abdominal cramps and headache. Persons at risk include infants, the elderly, and the immuno-compromised individuals in whose case complications lead to meningitis, septicemia, reiter syndrome and death (Pavia and Tauxe 1991).

Several *Salmonella* outbreaks have been associated with the consumption of cantaloupes. *Salmonella* outbreaks associated with the consumption of cantaloupes may have at its source from the soil during production. Therefore it is important to investigate the attachment and survival of pathogens on the surface of cantaloupes. Cantaloupe having a pH of 6.3 to 6.7 (Lund 1992) and high water activity of 0.97 to 0.99 is capable of supporting the growth of pathogens. Cantaloupes, containing sugars, fructose, glucose and sucrose as percent soluble solids from 18 to 14% (Lester and Dunlap 1985) along with near neutral pH make an ideal substrate for growth. Therefore it is important to eliminate pathogens before they enter the inner tissue of the cantaloupe. Since the source of contamination cannot be eliminated, the need to wash and sanitize the produce is considered an important decontamination tool. The produce industry usually uses water and chlorine to wash the produce. The use of chlorine to wash produce was found to be

no effective in case of produce with high organic matter. The activity of chlorine as a disinfectant is neutralized with high organic matter. Thus the need of other sanitizing agents as a decontamination tool has become important.

Lactic acid and ozone have been found to be good alternatives to decontaminate produce. Research studies indicate lactic acid as a good disinfectant to decontaminate beef carcasses (Castillo and others 2001). The use of ozone as an antimicrobial agent in food processing was reviewed by Kim and others (1999) and Xu (1999); however little has been reported about the inactivation of pathogens on produce. So this study aims at presenting laboratory experiments towards disinfecting *Salmonella* Poona on cantaloupes, using chlorine, lactic acid and ozone.

OBJECTIVES

1. To compare a rifampicin-resistant mutated strain of *S.* Poona to the parent strain to ensure accuracy of experimental results. 2. To compare the efficacy of different sanitizing treatments in reducing the bacteria on the surface of the cantaloupe. 3. To determine the transfer of *S.* Poona from the surface to the internal tissue during cutting. 4. To study the survival of *S.* Poona on fresh-cut tissue and change of pH over 15 days.

REVIEW OF LITERATURE

Food-borne Disease Outbreaks

Today food-borne diseases are a key concern of the food industry and US government. Bacteria, viruses, prions and parasites are some of the causes of the food-borne illness (Moreno- Lopez 2002). The reporting of food-borne diseases in the US began earlier than 60 years ago when the state health officials were concerned about the high morbidity and mortality caused by typhoid fever and infantile diarrhea caused by the consumption of milk (Public Health Service 1925). The surveillance data from the US Centers for Disease Control and Prevention (CDC) revealed that the food-borne diseases caused approximately 76 million illnesses, 325000 hospitalizations and 5000 deaths in each year (Mead and others 1999). Pathogens that are the main concern today are *Salmonella*, *Campylobacter jejuni*, *E. coli* O157:H7, *Listeria monocytogenes* and *Cyclospora cayetanensis*. The data implicated symptoms of food-borne illness include gastroenteritis, renal and hepatic disorder and neurological syndromes. Data published in 1999 states that each year about 386 million illnesses were caused by the known pathogens out of which about 13.8 million (36%) cases were food-borne. Out of these 13.8 million cases 13% were caused by bacteria, 7% by parasites and 80% by viruses. Most of the acute gastroenteritis cases reported are caused by food-borne transmission and bacterial pathogens contribute about 60%, parasites about 5% and viruses are responsible for about 34% of the hospitalizations (Mead and others 1999).

Food related deaths are of big concern and reports issued by CDC show that of all the pathogens that cause food-borne transmission, five pathogens responsible for

causing 90% of the deaths. They are *Salmonella*, *Listeria*, *Toxoplasma*, Norwalk-like viruses, *Campylobacter* and *E. coli* O157:H7. Of all the deaths *Salmonella* causes 31%, *Listeria* 28%, *Toxoplasma* cause about 21%, Norwalk-like viruses are responsible for 7%, *Campylobacter* for 5% and *E. coli* for about 3% of the deaths (Mead and others 1999).

Food-borne Outbreaks Associated with Fruits and Vegetables

Outbreaks of food-borne illness associated with consuming raw fruits and vegetables in the United States have occurred more frequently in recent years (Bean and Griffin 1990; Beuchat 1996; De Roever 1998; CDC 1999; CDC 2000). Factors contributing to these outbreaks include change in consumer dietary habits, insufficient knowledge of hygienic practices and shifts in social demographics (Hedberg and others 1994; Beuchat and Ryu 1997). Nutritionists emphasize the importance of fruits and vegetables to a healthy diet and researchers have recommended consumption of at least five servings per day (Kennedy and others 1996). Fruits and vegetables are also found to be good source of nutrients, rich in fiber and are also known to protect us against cancer and lessen the risk of coronary heart disease (Marcus and others 1998).

A wide variety of produce has been linked by epidemiologic investigations to food-borne outbreaks for nearly a century (Pixley 1913; Warry 1903). The CDC however has reported increased numbers of produce–associated outbreaks in the US during the period between 1988 and 1992 compared with previous surveillance records (National Advisory Committee on Microbiological Criteria for Foods 1999). Documented illness has been linked to bacteria, parasites and viruses (Beuchat 1996).

The viruses involved in outbreaks include Noro-virus and it was found to cause an outbreak in raspberries or frozen strawberries and the protozoa that have been mostly involved in outbreaks include *Cyclospora, Giardia* and *Cryptosporidium*. *Cyclospora* was found to cause an outbreak in raspberries and probably in blackberries imported from Guatemala (Herwaldt and Ackers 1997; Flemming and others 1998). Hepatitis A virus was traced in an outbreak in frozen strawberries in the US (CDC 1997a; Hutin and others 1999). Data provided by Wells and Butterfield (1997) indicate that *Salmonella* is more readily isolated from decaying fruits and vegetables. Outbreaks associated with raw fruits and vegetables and unpasteurized products between 1980 and 2000 are listed in Table 1.

Table 1- Outbreaks associated with raw fruits and vegetables and unpasteurized products between 1980-2000 (Beuchat 2002).

Microorganism	Location	Produce
Bacteria		
Bacillus cereus	USA	Seed Sprouts
Clostridium botulinum	USA	Cabbage
E. coli O157:H7	USA	Apple cider, Lettuce, Apple juice, Alfalfa Sprouts
	Japan	Radish sprouts
L. monocytogenes	USA	Celery, Lettuce, Tomato
	Canada	Cabbage
Salmonella		
Miami	USA	Watermelon
Typhimurium	USA	Apple cider
Oranienberg	USA	Watermelon
Chester	USA	Cantaloupes
Javiana	USA	Tomatoes
Poona	USA/Canada	Cantaloupes
Montevideo	USA	Tomatoes
Stanley	USA	Alfalfa Sprouts
Shigella sonnei	USA	Lettuce, Parsley
	Norway	Lettuce
Vibrio cholerae	USA	Coconut milk
Viruses		
Hepatitis A	USA	Lettuce, Tomatoes
Norwalk	Uk	Melons
	USA	Green salad, Celery
Parasites		
Cyclospora cayetanensis	USA	Lettuce, Raspberries
Cryptosporidium parvum	USA	Apple cider
Giardia lambia	USA	Raw vegetables

For example, an outbreak of *Salmonella* Newport infection in 1995 involving 133 cases in Oregon and British Columbia was traced to alfalfa sprouts produced from a single contaminated lot of seeds (Van Beneden and others 1999). The outbreaks associated with *E. coli* O157:H7 attributed to fresh produce include lettuce (Ackers and others 1998), alfalfa sprouts (CDC 1997b) and unpasteurized apple cider (Besser and others 1993; CDC 1996). *L. monocytogenes* is a particular food safety concern because it is widespread in the environment (Gellin and Broome 1989). *L. monocytogenes* was found to be the most prevalent disease causing microorganism soil (Welshimer 1960) and found to be isolated from soil, sewage, sludge and water (Farber and Peterkin 1991; Farber and others 1989) and therefore has the potential to contaminate cantaloupe surfaces. Nevertheless there have been reports of contamination of produce with a wide variety of pathogens. *Salmonella* is the most frequently reported cause of food borne outbreaks of gastroenteritis in the United States (Mead and others 1999). *Salmonella* spp are estimated to cause approximately 1.5 million cases of food borne infection each year in the US with more than 15000 hospitalizations and 500 deaths (Mead and others 1999). Out of the total 30 deaths caused by food borne contamination in the United States, 95% deaths occur due to *Salmonella* infection (Santos and others 2003). In recent years, a large number of *Salmonella*-related outbreaks have been found to be in association with the consumption of contaminated fruits and vegetables especially with tomatoes, melons, orange juice, apple juice and sprouts (Burnett and others 2000). In the years 1990 and 1993 outbreaks of *Salmonella* Javiana (Wood and others 1991) and *Salmonella* Montevideo (CDC 1993) infections involving 170 and 100 cases respectively in Illinois,

Michigan, Minnesota and Wisconsin were epidemiologically linked to the consumption of fresh tomatoes. In 1995, an outbreak of *Salmonella* Hartford, *Salmonella* Gaminara and *Salmonella* Rubislaw infections occurred among 62 unrelated persons at a theme park in Orlando, Florida (CDC 1995) and investigations revealed that the illness was associated with the consumption of orange juice. In 1995, an outbreak of *Salmonella* Stanley infection involving 23 states in US was linked to the consumption of alfalfa sprouts (Mohon and others 1997).

Attachment of Pathogens to the Surface of Fruits and Vegetables

The ability of pathogenic bacteria to adhere to surfaces of fruits and vegetables continues to be a potential food safety problem of great concern to the food industry. Fruits and vegetables are usually in contact with soil, insects, animals and humans during growing, harvesting (Trueman 1971) and in the processing plant (Mudrock and Brokaw 1957). Thus the surfaces are not free from natural contaminants and by the time they reach the packing house, most of the produce retains populations of 10^4 to 10^6 cells/g (Brackett 1992; Beuchat 1996). The attachment of bacteria on the surface of fruits and vegetables is governed by a number of factors such as pH of the fruit, water activity and the medium in which the bacteria are grown (Iturriaga and others 2003). The attachment of bacteria on the surface of fruits is not clear; however, research indicates that it was similar to the attachment of plant pathogenic bacteria (Iturriaga and others 2003). The flagella, fimbriae and proteins on the outer membranes may influence the bacterial attachment to the plant surfaces (Romantschuk 1992; Strom and Lory 1993).

The plant surfaces and the microbes, both having negative surface charge, exhibit electrostatic repulsion between the surfaces. But the fimbriae present on the surface helps to bridge the gap exerted by the electrostatic repulsion (Romantschuk and others 1996).

When bacteria attach to the surfaces of fruits and vegetables, they tend to locate in pores, indentations or other natural irregularities on the intact surfaces where there are protected binding sites (Seo and Frank 1999). Bacteria also attach to the cut surfaces (Liao and Cooke 2001) or in punctures and cracks in the commodity surface (Burnett and others 2000). There are many reports of disease caused by the consumption of fruits and vegetables that were contaminated at the surface with enteric pathogens (Beuchat 1996; Geldreich and Bordner 1971). A number of commodities (apples, pears, cherries, grapes, zucchini squash, potatoes, carrots and lettuce) often have punctures, cuts or splits that could be the sites for bacterial attachment. *Salmonella* Chester was found to survive after washing when attached to cut surfaces of apple and green pepper disks than on unbroken external surfaces (Seo and Frank 1996; Liao and Sapers 2000). Growth of *E. coli* within punctures was demonstrated in artificially inoculated apples in spite of the fruit having high acidity (Sapers and others 2000). It has been demonstrated that the application of bacteria to the surface of fruits will result in their internalization over time (Samish and others 1963).

Survival of Pathogens on the Surface of Fruits and Vegetables

The survival and growth of pathogens on fresh produce is influenced by the organism, produce item and environmental conditions in the field, including storage conditions (FDA 2001). Association of the pathogens with fruits depends on many factors such as the environment in which plants are grown, pH of the tissue and the presence of antimicrobial factors on the surface or inside of the fruits (Burnett and others 2000). Growth of pathogens on the intact surfaces of the fruits is not common because of the incapability of food-borne pathogens to produce enzymes which are necessary to breakdown the protective barriers. This restricts the availability of nutrients and moisture. But previous studies indicate growth of *E. coli* O157:H7 on the surface of watermelon and cantaloupe rinds (Van Loosdrecht and others 1987). Proliferation of post harvest microorganisms may compromise the peel integrity and alter the product pH, thereby enhancing the survival and growth of human pathogens (Conway and others 2000).

pH is an important factor since the bacteria are inactivated at lower pH values. Many fruits (apples, oranges) are more acidic and do not support the growth of human pathogens. A number of melons and soft fruits have pH values that are substantially higher than 5.0 and will support the growth of pathogenic bacteria (Nguyen-the and Carlin 1994; Del Rosario and Beuchat 1995; Beuchat 1996). Tomatoes are of particular interest because there is a misconception that they do not support the growth of pathogenic bacteria since the pH of tomatoes range from 3.4-4.8 (Lund 1992). Studies indicate that *Salmonella* can grow on sliced or cut tomatoes at pH values as low as 3.99

(Asplund and Nurmi 1991; Wei and others 1995; Zhuang and others 1995). Storage temperature becomes a critical factor for the produce whose pH permits the growth of pathogenic bacteria. Also, we now know that pathogens are usually not killed by the low pH of fruits and some vegetables.

Temperature plays an important role in the survival of pathogens on the surface of fruits and vegetables (Gawande and Bhagwat 2002). There can be an exponential multiplication of bacteria when temperatures are increased and humid conditions are maintained (Splittstoesser 1970). Previous studies also indicate that *E. coli* O157:H7 in broccoli, cucumber and green pepper could survive when held at 4°C and maintain initial levels or grow at 15 °C (Richert and others 2000). While growth of pathogens may be inhibited by chilled temperatures, survival can be enhanced under certain conditions. For example *Salmonella* and *E. coli* O157:H7 survive for a longer period in fruit juices under refrigeration than at room temperature (Parish and others 1997). The temperature of water used during the handling of produce plays an important role on the safety of fresh produce. Immersion of whole warm fruit in cool solution results in the internalization of the solution along with the pathogens, if any, into the fruit thus affecting quality and safety. Zhaung and others (1995) showed that *Salmonella* Montevideo is filtered into the core of tomatoes when there is a temperature differential of 15 °C.

Surface contact is also known to affect the survival of pathogens and studies show that as compared with free cell suspension, *Salmonella* exhibits more resistance to antimicrobial agents and temperature abuse when the bacteria is attached to the surface

(Dhir and Dodd 1995). It was also indicated that plant tissues have naturally occurring antimicrobials that provide varying levels of protection against the growth of pathogens (Lund 1992). The studies conducted on the effect of growth phase on the survival of pathogenic bacteria indicate that growth phase initiates some phenotypic and genotypic responses in bacteria that support the survival of pathogens under stress conditions (Taylor and others 2003).

Incidence of *Salmonella* in Cantaloupes

As discussed earlier *Salmonella* is the most frequently reported case of food-borne outbreaks of gastroenteritis in the United States. Salmonellosis, a major food-borne infection was found to be associated with the consumption of cantaloupes which were contaminated by various serovars of *Salmonella.*

In the US, a large multi-state outbreak leading to 245 confirmed cases of *Salmonella* serotype Chester was traced to the consumption of cantaloupes in 1990. Apparently transmission of pathogens to the interior of the cantaloupe may have occurred while cutting the unwashed melons (Beuchat 1996). In another outbreak, more than 400 cases were reported from 23 states in the US and two provinces in Canada. The causative agent of the illness in this outbreak was *Salmonella* serotype Poona linked to the consumption of contaminated cantaloupes produced in Texas (CDC 1991). Another outbreak with more than 20 cases was reported in California due to *Salmonella* Saphra in 1997 (Los Angeles County Department of Health Services- Public Health 2001).

Another outbreak involving about 22 cases was reported in Ontario in 1998 by *Salmonella enterica* Oranienburg (Deeks and others 1998).

Furthermore, there were three multi state outbreaks of *Salmonella* Poona infections associated with eating cantaloupe imported from Mexico between 2000 and 2002. These three outbreaks caused two deaths and 18 hospitalizations and each outbreak involved 35-50 illness cases (CDC 2002).

From the above data, most of the cases of salmonellosis in cantaloupes were caused by *Salmonella* Poona. *Salmonella* Poona is an organism which can cause serious and sometimes fatal infections in young children, elderly people and others with weakened immune system. Persons infected with *Salmonella* Poona often experience fever, diarrhea, nausea, vomiting and abdominal pain. In rare circumstances, infection can result in the organism getting into the blood stream and producing more severe illnesses such as arterial infections endocarditic and arthritis.

FDA inspections revealed that these outbreaks between 2000 and 2002 were due to insanitary conditions in the growing and packing of cantaloupe in Mexico. It also said that *Salmonella* contamination may include irrigation with sewage-contaminated water, cleaning and cooling with contaminated water, poor hygiene practices by processing workers and inadequate cleaning of the equipment used for handling of the product (Center for Infectious Disease Research and Policy, CIDRAP 2002). So in 2002, the US Food and Drug Administration (FDA) issued an import alert on cantaloupes from Mexico until it can certify that these cantaloupes are produced under sanitary conditions (CFSAN 2002).

Salmonella Contamination on Cantaloupes

Cantaloupe normally grown on the ground, in contact with the soil, can be contaminated with a human pathogen such as *Salmonella* anytime during production. Surface structure and biochemical properties of and of a substratum play a major role in how and where the bacteria may attach. The surface of cantaloupe is composed of a meshwork of tissue commonly referred to as net (Webster and Craig 1976). Elaboration of this net tissue has been characterized as a response to cracking of the fruit surface (Meissner 1952). This raised net tissue gives the surface of the cantaloupe an inherent roughness. This surface roughness may favor bacterial attachment and hinder microbial detachment.

Bacterial attachment to the surface is influenced not only by cell surface charge (Fletcher and Loeb 1979) and hydrophobicity (Van Loosdrecht and others 1987; Vander Mei and others 1991) but also by the presence of particular surface appendages such as flagella, fimbriae and as well as extra cellular polysaccharides (Frank 2000). Research indicates that bacterial attachment to fruits may be similar to the attachment of plant pathogenic bacteria (Iturriaga and others 2003). Plant pathogenic bacteria attach to the surface through both reversible and irreversible attachment, which involves the weak vander wall force of attraction between the cells and the surface (Iturriaga and others 2003). The surface of the cantaloupe may get damaged during picking or shipping and thus provide access to the nutrients of the tissue and survive. Survival of *Salmonella* on the surface and scar portions of the cantaloupe was observed. Laboratory studies revealed that *Salmonella* Stanley survived on the surface of the cantaloupe at 20 °C and

4 °C for up to 144 h (Ukuku and Sapers 2001). The survived bacteria on the surface may get internalized into the tissue through stomata, lenticels, broken trichomes and bruises and cracks in the skin surface of fruits and vegetables (Wells and Butterfield 1997). The natural biological barrier of cantaloupes provides some protection against the invasion of pathogens until this barrier is compromised by slicing and cutting. Cantaloupe having a pH of 6.3 to 6.7 (Lund 1992) and high water activity of 0.97 to 0.99 is capable of supporting the growth of pathogens. Cantaloupes containing sugars fructose, glucose and sucrose as percent soluble solids from 18 to 14% (Lester and Dunlap 1985) along with near neutral pH make this fruit ideal substrate for growth. The growth of pathogens is accelerated when the temperature is made favorable. Golden and coworkers (1993) found that *Salmonella* grew rapidly on the cut surfaces of cantaloupe, watermelon and honey dew melon that were held at room temperature. *Salmonella* levels remain unchanged when the melons were held at refrigerated temperatures. Studies indicate that *Salmonella* will survive at refrigerated temperatures but is capable of growing when the temperature is made favorable. Ukuku and Sapers (2001) reported the growth of *Salmonella* Stanley on cantaloupe cut pieces stored at 20 °C or higher and observed a growth of 2-4 log CFU/g in 6 h. *L. monocytogenes* is also capable of surviving in cut pieces during long term storage (5 days) at 4 °C (Ukuku and Fett 2002).

FDA conducted field surveys on imported melons during 1990 and 1991. Results of the survey revealed that in 1990 and 1991, 0.76 and 1.06 % of the melon rinds respectively harbored a variety of *Salmonella* spp on their surfaces. As the melons grow on the ground and are exposed to microorganisms, they have the potential to become

contaminated with pathogens. The association of illness with salad bars and fruit salads suggests that *Salmonellae* may have been introduced into the fruit from the rind by the physical act of cutting the melon or contact by cut pieces of melon with contaminated rinds. Besides, the contaminated fruit may have remained at room temperature for several hours after preparation (CDC 1991).

Since minimally processed fruits such as cantaloupe can be contaminated by pathogens like *Salmonella* and as there is no heating or kill step involved in the processing of these fruits and fresh cut products and are typically consumed raw, the need for intervention methods to maintain safety is very important.

Decontamination

It is well known that fruits and vegetables are associated with disease causing outbreaks due to presence or contamination of pathogenic bacteria. So this renders a need for improved mitigation efforts to reduce risks associated with these products.

There are a variety of methods to reduce populations of microorganisms on whole and fresh cut produce. To minimize the risk of infection associated with raw fruits and vegetables, potential sources of contamination from the environment to the table should be identified and eliminated. Since the source of contamination cannot be eliminated, the need to wash and sanitize the produce is considered as an important decontamination tool.

FDA Guide to minimize microbial safety hazards for fruits and vegetables (FDA, 1998) suggested a definition of sanitizer which is to treat clean produce by a process that

is effective in destroying or substantially reducing the numbers of microorganisms of public health concern, as well as other undesirable microorganisms, without adversely affecting the quality of the product or its safety for the consumer.

Several types of treatment are known to be partially effective in removing disease-causing microorganisms from the surface of whole and cut raw fruits and vegetables or from contact surfaces during handling. None of the treatments except irradiation using chemical agents can be relied upon to totally disinfect raw produce when applied in levels that do not cause deterioration in sensory quality. Even irradiation is not effective in killing viruses on fruits and vegetables. Rather, these treatments should be considered as methods of disinfection causing reductions in populations of microorganisms but not completely removing pathogens from fruits and vegetables.

Each type of disinfectant has its own efficacy in killing microorganisms. The resistance of microorganisms to the disinfectant depends on the type, pH of the disinfectant, contact time, temperature and the chemical and physical properties of fruits and vegetable surface. Very little is known about the efficacy of disinfectants in relation to the roughness of fruit surface, although higher amount of cuticle may protect against the penetration of cells, thereby increasing the need for exposure to chemical treatments. There are many chemical sanitizers approved as Generally Recorded as Safe (GRAS) chemicals but our study mainly focuses on chlorine, lactic acid and ozone.

Chlorine

Chlorine has been used for sanitation purposes in food processing for several decades and is perhaps the mostly used sanitizer in the food industry (Cherry 1999). Chlorine is commonly used at concentrations of 50-200 ppm with a contact time of 1-2 min to sanitize produce surfaces (CFSAN/FDA 2001). The ability of chlorine to destroy microorganisms depends on the amount of free residual chlorine i.e. chlorine remaining after it reacts with organic matter, in the water (Gavin and Weddig 1995).

Although Sodium hypochlorite is used to treat the produce, it is the resulting hypochlorous acid that exerts the lethal effect upon the microorganisms.

$$Cl_2 + H_2O \rightarrow HOCl + HCl.$$

The lethal effects of chlorine were observed at pH range of 6.0-7.5 (Sapers 2003). When clorox (Sodium hypochlorite) is added to water, the pH increases. Raising the pH of the solution will result in lowering the amount of chlorine that is available as hypochlorous acid (HOCl). If pH increases above 8 the hypochlorous acid splits to form hydrogen ions (H^+) and hypochlorite (OCl^-) ions.

$$HOCl \rightarrow H^+ + OCl^-$$

This hypochlorite ion thus formed has weak bactericidal effects. So the pH of the solution is adjusted by the addition of organic or inorganic acids to lower the pH. Studies have shown the usefulness of acidic hypochlorite solution adjusted to pH 5.0 with hydrochloric acid as powerful and economical disinfectant (Iwasa and Nakamura 1996; Okubo and others 1998). The main advantages of using this chlorine as a sanitizing agent are relatively inexpensive and wide action against many micro-organisms. But

recently concerns have been raised about its use due to the formation of chlorinated by-products (Richardson and others 1998).

Effects of chlorine on bacterial pathogens inoculated onto produce have been investigated. Populations of *Salmonellae* or *E. coli* O157: H7 inoculated onto the surface of cantaloupes and honeydew melons were reduced between 2.6 and 3.8 log CFU (as compared with water wash control) when treated for 3 min with 2000 ppm sodium hypo chlorite or 1200 ppm acidified sodium chlorite (Park and Beuchat 1999). These treatments were less effective when applied to asparagus.

Research reported by Nguyen-the and Carlin (1994) suggested that removal of *L. monocytogenes* on vegetables by chlorine is limited. Zhang and Farber (1996) showed that the treatment of shredded lettuce and cabbage with 200 ppm chlorine for 10min reduced populations of *L. monocytogenes* by 1.7 and 1.2 log CFU/g respectively. When inoculated into cracks of mature green tomatoes, *Salmonella* Montevideo survived treatment with 100 ppm chlorine (Wei and others 1995).

Based on the data from previous studies, it is necessary to customize sanitation treatments for different types of produce. Since chlorine reacts with organic matter, components leaching from tissues of cut produce surfaces may neutralize some of the chlorine before it reaches microbial cells, there by reducing its effectiveness. Failure to maintain adequate chlorine in wash solutions may lead to increase in microbial populations on fruits and vegetables (Beuchat 1999). When considering this phenomenon, it is apparent that decontamination methods besides chlorine rinses are needed to ensure safety of produce.

Organic acids

Organic acids naturally present in fruits and vegetables or which are accumulated as a result of fermentation are relied upon to retard the growth of some micro-organisms and prevent the growth of others. Food borne bacteria which are capable of causing human illness cannot grow at pH values of about 4.0 so; the acidic pH of edible portions of most fruits precludes them as substrates for proliferation of human pathogens. However the pH of many vegetables and few fruits especially melons is in the range at which pathogens can grow. The antimicrobial mechanism of organic acids has not been completely understood. However it is believed that the microbial inhibition by weak acids is not solely due to the creation of high extra cellular proton concentration, but is also directly related to the concentration of undissociated acid (Adams and Moss 2000). The weak acids in their undissociated form can pass freely through the cell membrane and accumulate in the cytoplasm. If the intracellular pH is high, the undissociated acid ionizes produce protons and acidifies the cytoplasm of the microorganism. Thus if the external pH is low and the extra cellular concentration of acid is high, the cytoplasmic pH drops to a level where growth is no longer possible and the cell eventually dies.

Washes and sprays containing organic acids, particularly lactic acid, have been successfully used to disinfect beef, lamb, pork and poultry carcasses. Applications of organic acid washes, particularly lactic acid to the surface of fruits and vegetables for the purpose of reducing populations of microorganisms have not been extremely reported. The effects of lactic acid and acetic acid, either alone or in combination with chlorine, on survival of *L. monocytogene*s inoculated onto shredded lettuce were studied by Zhang

and Farber (1996). Compared with lettuce washed in tap water, only 1% lactic acid and combinations of 0.5% or 1% lactic acid and 100 ppm chlorine reduced numbers of *L. monocytogenes*. Lactic acid (0.75% or 1%) in combination with 100 ppm chlorine was more effective in reducing levels of *Listeria* than chlorine alone. Results obtained by acetic acid were similar to those of lactic acid.

Studies indicate that pathogenic microorganisms on the surface of uncut fruits and vegetables after washing with water or a disinfectant solution, or transferred to the flesh or pulp of fruits and vegetables during cutting process , can be killed or prevented from growing by applying organic acids. Escartin and others (1989) reported that application of lemon juice to the surface of papaya inoculated with *Salmonella* Typhi reduced populations compared with control, but growth resumed after several hours. Survival of *Clostridium jejuni* inoculated onto watermelon and papaya cubes as affected by treatment with lemon juice was investigated by Castillo and Escartin (1994).

Removal of pathogenic bacteria from leafy salad greens by treating with acetic acid has been studied. Reduction in counts of *Yersinia enterolitica* inoculated onto parsley leaves from 10^7 CFU/g to < 1 CFU/g by washing in a solution of 2% acetic acid or 40% vinegar for 15 min was achieved by Karapinar and Gonul (1992).

Studies on the efficacy of per acetic acid in reducing microbial counts in produce wash water and on fruit surfaces were reported (Hei 1998). Sanitizers using per acetic acid at 40-80 ppm significantly reduced populations of *Salmonella* and *E. coli* O157:H7 on cantaloupe and honey dew melon (Park and Beuchat 1999).

Ozone

Ozone is a strong antimicrobial agent with numerous potential applications in food industry. High reactivity, penetrability and spontaneous decomposition to a nontoxic product (i.e. O_2) make ozone a viable disinfectant for ensuring the microbial safety of food products. The use of ozone as an antimicrobial agent in food processing was reviewed by Kim and others (1999) and Xu (1999); however, little has been reported about the inactivation of pathogens on produce.

In 1982, the US FDA affirmed ozone as GRAS with specific limitations, for use as a disinfectant in bottled water (FDA 1982). In 2001, gaseous aqueous ozone was approved by USFDA for application as an antimicrobial agent to foods (FDA 2001).

Giese and Christenser (1954) suggested that the bactericidal cell surface is the primary target of ozone activity. Scott and Lesher (1963) detected the leakage of cell contents with ozone treatment. They proposed the double bonds of unsaturated lipids in the cell envelope as the primary site of attack. Murray and others (1965) assumed that lipophilic and lipopolysaccharide layers of gram negative bacteria would be subjected to attack by ozone that results in a change in cell permeability, eventually leading to lysis.

Ozone may inactivate microorganisms by causing damage to their genetic material. In studies by Prat and others (1968) and Scott (1975) on DNA of *E. coli*, the pyrimidine bases were modified by ozonation, with thymine being more sensitive to ozone than cytosine and uracil. Ozone is an effective treatment for drinking water and will inactivate bacteria, fungi, viruses and protozoa (Peters and others 1989; Korich and others 1990). According to Restaino and others (1995), bacterial pathogens such as

Salmonella Typhimurium, *Y. enterolitica, S .aureus* and *L. monocytogenes* are sensitive to treatment with 20 ppm ozone in water. Treatment with ozonated water can extend the shelf life of apples, grapes, oranges, pears, raspberries and strawberries by reducing microbial populations and by oxidation of ethylene to retard ripening (Beuchat 1998). Microbial populations on berries and oranges were reduced by treatment with 2-3 ppm and 40 ppm respectively. Kim and others (1998) reported a 2 log/g reduction in total counts for shredded lettuce suspended in water ozonated with 1.3 mM ozone at flow rate of 0.5 L/min.

In contrast to use of ozone as an initial treatment to reduce microbial populations on the produce surface, ozone gas has also been investigated for use a sanitizer on poultry (Sheldon and Brown 1986), pork, beef, dairy products, eggs, mushrooms, potatoes and fruits (Kaess and Weidemann 1968; Gammon and Kerelak 1973). Apples stored in an atmosphere containing ozone had reduced incidents of spoilage (Bazarova 1982). Treatment of grapes by ozone increased shelf life and reduced fungal growth (Sarig and others 1996). Due to the strong oxidizing activity, ozone may cause physiological injury to the produce (Horvath and others 1985). Bananas treated with ozone developed black spots after 8 days of exposure to 25 to 30 ppm gaseous ozone. Carrots exposed to ozone gas during storage had a lighter, less intense color than untreated carrots (Liew and Prange 1994). However much research was not done on the effectiveness of ozone as a sanitizer on cantaloupes and thus a need to observe the efficacy of ozone on rough surfaces led to the investigation of this research.

Ozone was also tested as an alternative to chlorine because of its various advantages over chlorine as a sanitizer. In evaluating ozone as a potential alternative to chlorine, the Food Manufacturing Coalition (1996) stated: “Any new technology as effective as chlorinated solutions in reducing microbial (especially bacterial) contamination and able to meet USDA standards for microbial count reduction in the particular industry, whether poultry, beef or other”. Much information attesting the superiority of ozone over other chemical disinfectants was studied. Use of chlorine is mainly dependent on pH since efficacy of chlorine diminishes as pH increases whereas ozone potency is not affected very much by pH but ozone decomposition occurs at high pH. However ozone treatment is considered more expensive than chlorine, improvements in ozone generators, better controls, increasing concerns about the hazards of storing large supplies of toxic chlorine gas in high population areas, the handling and disposal of corrosive chemicals required for on-site generation of chlorine and the safety concerns about the organic chlorine by-products argue strongly for consideration of ozone, on a chlorine-equivalency basis, as a safer alternative disinfectant.

The present study focuses on exploring the uses of lactic acid and ozone as a decontamination tools on the surface of cantaloupes. Lactic acid and ozone were also tested for their efficacy in reducing the transfer of *Salmonella* from the surface to the fresh cut tissue.

MATERIALS AND METHODS

Bacterial Culture

The rifampicin resistant strain and the parent strain of *Salmonella* Poona provided by Dr. Linda Harris, from the University of California, Davis was stored at -80 °C on Protect™ Bacterial Preservers (Key Scientific Products, Round Rock, Texas). The microorganism was resuscitated by 2 successive transfers to Tryptic Soy Broth (TSB; Difco) and incubated at 37 °C for 18-24 h. The cells were then transferred on to the Tryptic Soy Agar (TSA; Difco, Detroit, MI) slants and were stored approximately at 4-5 °C until they were needed for the study. Prior to inoculation, rifampicin-resistant cultures were confirmed by streaking onto TSA plates supplemented with 80 µg/ml rifampicin (Sigma, St Louis, MO; RIF-TSA) and incubating at 37 ºC for 24 h. Characteristic colonies were then streaked onto TSA slants and incubated at 37 ºC for 24 h. Then during the day before the actual experiments, the organism was transferred into a glass tube containing 9 ml of TSB and incubated overnight at 37 °C for 18-24 h. The tube with an overnight growth contained approximately 8 $\log_{10}$ CFU/ml.

Preliminary Studies

The organism used is a rifampicin strain of *S.* Poona, so different preliminary tests were conducted in order to confirm that the rifampicin resistant strain was virtually indistinguishable from the parent strain. The preliminary studies include a) Growth curve characteristics b) Heat tolerance and c) Sensitivity towards lactic acid.

Growth curve characteristics

During the day of actual experiment, the cells were then subjected to two successive transfers each of 1 ml into glass tubes containing 9 ml of TSB making the final concentration of approximately 6 $\log_{10}$ CFU/ml. Then a final transfer of 0.1 ml was made into several of the glass tubes containing 9 ml of TSB and incubated at 37 °C. The experiment was done in triplicate. The tests for the growth of the organism were observed for every hour for up to a period of 12 h and a final count was conducted after 24 h of incubation. For every 1 hr, 3 tubes were removed at random to find the counts of the organism. The numbers were determined from each tube (sample) by surface plating of 0.1 ml of serial ten-fold dilutions of 0.1% peptone (Difco) onto the RIF-TSA plates followed by incubation at 37 °C for 18-24 h. The same procedure was conducted with parent strain of *S.* Poona also and the growth curves of the rifampicin resistant strain of *Salmonella* and that of the parent strain were compared.

Heat tolerance study

The parent strain and the rifampicin resistant strain of *S.* Poona were tested for their resistance to heat at 50 °C and 60 °C. On the day of the experiment the cultures containing approximately 8 $\log_{10}$ CFU/ml were harvested by centrifugation at 3500 rpm for 10 min in a Jouan Centrifuge model B4 (Winchester, VA). The cell pellets were recentrifuged twice in 9.9 ml of 0.85% sterile saline solution. They were finally suspended in the saline solution. 0.5 ml of the suspension was transferred into several 17 x 60 mm screw cap vials containing 4.5 ml of 0.85% sterile saline solution. The screw cap vials (held upright by a sponge rack) were heated in a water bath to 50 °C or 60 °C

and the temperature of the vials was measured, using a K-type thermocouple connected to a Traceable® 2-channel hand-held digital thermometer (Control Company, Friendswood, TX) which was placed in a non inoculated vial containing 5 ml of 0.85% sterile saline. As the temperature recording on the thermocouple reached 50 °C, three vials were removed at random and placed in a beaker containing ice water. At intervals of 30, 60, 90 and 120 s, another 3 vials were randomly separated and immediately placed in ice water. Three vials containing the pure culture of each strain were used as the control and were plated on TSA plates to determine the original concentration of both strains. All vials were removed from the ice water and tested for bacterial count by plating serial dilutions onto RIF-TSA plates and onto TSA plates supplemented with 2% NaCl (Sigma, TSA+NaCl). The lethal effect of heat was determined by observing the survival of the organism at different time intervals, whereas the sublethal effect was determined by subtracting the count obtained on TSA+NaCl from the count on RIF-TSA. Thus the curves drawn from both the organisms were used to compare the effect of heat on rifampicin strain of *S.* Poona with that of the parent strain.

Lactic acid resistance

The sensitivity of the parent strain and the rifampicin-resistant strain of *S.* Poona to lactic acid was conducted by placing both the organisms in 1% lactic acid solution. A tube with an overnight growth contained approximately 8 $\log_{10}$ CFU/ml of each organism was centrifuged and the cells were then harvested by centrifugation at 3500 rpm for 10 min and the pellets were suspended in 0.1% peptone water. The cells were centrifuged again at 3500 rpm for 10 min and the pellets were resuspended in 9 ml of

0.1% peptone to wash the cells. The inoculum prepared was serially diluted in 0.1% peptone water and 1ml of the culture having approximately 6 $\log_{10}$ CFU/ml was transferred to several tubes, each containing 9 ml of 1% lactic acid solution at room temperature making a final concentration of 5 $\log_{10}$ CFU/ml. 1% lactic acid solution was prepared by diluting the 88% L-lactic acid (Purac, Lincolnshire, IL) with distilled water. The lactic acid resistance of the organisms was determined by plating the samples at different time intervals of 0, 30, 60, 90 and 120 s. The counts were determined by serial dilutions in 9 ml of 0.1% peptone water and spread plating a portion (0.1 ml) of the dilution onto the TSA plates and also on the TSA plates supplemented with 2% NaCl. The plates were incubated at 37 °C for 18-24 h. The experiment was done in triplicate. The stress undergone by the organisms were also determined by the difference between the counts obtained by plating on TSA+NaCl and the counts obtained by plating on TSA plates alone. Thus the curves drawn from both the organisms were used to compare the effect of lactic acid on rifampicin strain of *S.* Poona with that of the parent strain.

Survival of *Salmonella* on the surface of cantaloupe with rotation vs. stationary drying

Preliminary studies were also conducted to determine whether rotating the melons upside down for every 2 h in a 12 hr period of draining had any impact on the number of bacteria present on the overall surface and on the stem scar portion of the cantaloupes as compared to stationary draining. During the day before the experiment, the *S.* Poona culture was transferred into a flask containing 600 ml TSB and incubated at 37 °C for 18-24 h. The tube with an overnight growth contained approximately 8 $\log_{10}$ CFU/ml. The cells were then harvested twice by centrifugation at 3500 rpm for 10 min.

The cell pellets were finally suspended in 600 ml of 0.1 % peptone water. These cells were diluted in 5400 ml of 0.1% peptone water to make a 6 L of final inoculum. The final concentration of the inoculum was found to be 7 $\log_{10}$ CFU/ml. Twenty-four cantaloupes free from any bruises, having similar extent of network purchased from the grocery store, were inoculated by dipping them into the 6 L suspension of the culture and were manually rotated to ensure even inoculation. They were inoculated for 3 min. Upon removal of the cantaloupes from the bacterial suspension, the melons were held over the beaker until it ceased to drip the liquid and then they were placed on the racks which were placed inside a plastic tub. They were left to dry at room temperature for 12 h. The melons were placed on the trays in such a way that the scar portions of the cantaloupe were visible. For every 2 h of drying, 12 melons were manually rotated in such a way that the melons stand on the scar portions of the cantaloupe and the rest of the melons were left unrotated. Thus after 12 h of drying, two samples each of 10 cm^2 area were taken from the overall surface and one sample from the scar portion using a sterile stainless steel core borer. The borer was sterilized by dipping in 95% ethanol and flaming. It was used to initially cut the surface of the inoculated melon and a sterile scalpel and forceps were used to slice and remove the skin of the cantaloupe of approximately 1 mm – 2 mm deep. Both scalpel and forceps were sterilized between samples using 95% ethanol and flaming. The samples from the rind and the sample from the stem scar were placed in whirl-pak® bags (Nasco, Fort Atkinson, WI) each containing 90 ml of sterile 0.1% peptone water. The samples were then homogenized using a Stomacher-400 laboratory blender (Seward Scientific, London, England) for 1

min at normal speed. Viable counts were obtained by plating 0.1 ml of the homogenized sample onto the RIF-TSA plates followed by incubation at 37 °C for 18-24 h.

Cantaloupe Collection

Fresh untreated cantaloupes were obtained from a local distributor, Country Fresh Products, Houston, Texas and were brought to the Food Microbiology Laboratory in Texas A&M University, College Station, Texas. The Cantaloupes obtained were free from any visual defects such as bruises, cuts or abrasions and were of similar maturity levels and were also similar in the extent of their netting. A total of 120 melons were used in the study and these melons were shipped in two different batches and it was made sure that the melons obtained at different timings were of the same origin. A total of 18 melons were used for one treatment. The remaining melons which were not used during the first day were stored in cardboard boxes and kept under refrigeration temperatures (4 °C) until needed. The melons were brought to ambient temperatures by leaving them overnight at room temperature prior to inoculation.

Inoculum Preparation

The rifampicin resistant strain of *S.* Poona obtained from the University of California at Davis was used in the study. The strain was stored at – 80 °C. The microorganism was resuscitated by a successive transfer to TSB and incubated at 37 °C for 18-24 h. The cells were then transferred on to the TSA slants and were stored approximately at 4-5 °C until needed for the study. Then during the day before the actual experiment, the culture was transferred into a flask containing 600 ml of TSB, and incubated overnight at 37 °C for 18-24 h. This flask with an overnight growth contained

approximately 8 $\log_{10}$ CFU/ml. These cells were harvested by centrifugation at 3500 rpm for 10 min. The cell pellets obtained were washed twice in 0.1% peptone water. Finally this 600 ml suspension was transferred into a beaker containing 5400 ml of 0.1% peptone water making a total of 6 L inoculum. This suspension served as the inoculum for the test-cantaloupes. The final concentration of the inoculum was found to be 7 $\log_{10}$ CFU/ml as determined by plating on RIF-TSA plates followed by incubation at 37 °C for 18-24 h.

Cantaloupe Inoculation

The cantaloupes were inoculated by submerging them in a beaker containing the 6 L of the bacterial inoculum for 3 min, gently agitating with a glass rod to ensure even inoculation of the melon. The glass rod used was sterilized between inoculations by dipping in 95% alcohol and flaming. Upon removal of the cantaloupes from the bacterial suspension, they were held over the beaker until it ceased to drip the liquid and then they were placed on the racks inside a plastic tub. The melons were left to dry at room temperature for 12 h. After 12 h of drying, the cantaloupes were subjected to different surface sanitizing treatments.

Preparation of Sanitizing Solutions

Bleach (Clorox) containing 60% Sodium hypochlorite was used as the source to obtain the chlorine solution. Chlorinated water was prepared by diluting the bleach with distilled water so that it attains a concentration of 200 ppm free chlorine. The amount of free chlorine present in the solution was determined by using a Hach chlorine test kit (Hach Company, Loveland, Colorado, USA). The pH of the solution was adjusted and

brought to 6.5±.2 by using 1% Hydrochloric acid (EM Science, NJ) and was measured by using a Orion Model 230A pH meter (Orion Research INC, Boston, USA). 88% of L-lactic acid was used to prepare 2% of the L-lactic acid solution. It was also made by diluting the 88% L-lactic acid with distilled water. The temperature of the solution prepared was maintained to be 55 °C at the time of spray.

The ozone used in the study was obtained with a Lynntech-manufactured ozone generation system (Lynntech, College Station, TX). The ozone was produced electrochemically, using electric power and water as their sources. The reactions given below are made to occur by applying DC electricity between the anode and cathode which are positioned on either side of a Nafion 117 proton-exchange membrane as shown in Figure 1. When water is fed to the anode side, where the electrode is coated with lead dioxide; two water oxidation reactions take place; the oxygen (O_2) evolution reaction, equation (1), and the ozone (O_3), formation, equation (2).

$$2H_2O \rightarrow O_2 + 4H^+ + 4e^- \quad E^\circ = 1.23V \qquad (1)$$

$$3H_2O \rightarrow O_3 + 6H^+ + 6e^- \quad E^\circ = 1.51V \qquad (2)$$

Utilization of high over potentials (i.e., anode potentials much higher than 1.23 Volts) and certain electrode materials (e.g., lead dioxide) enhances O_3 formation at the expense of O_2 evolution. The O_3 and O_2 partition between the liquid and the gas phases as they are formed. The water oxidation reactions yield protons and electrons, which are

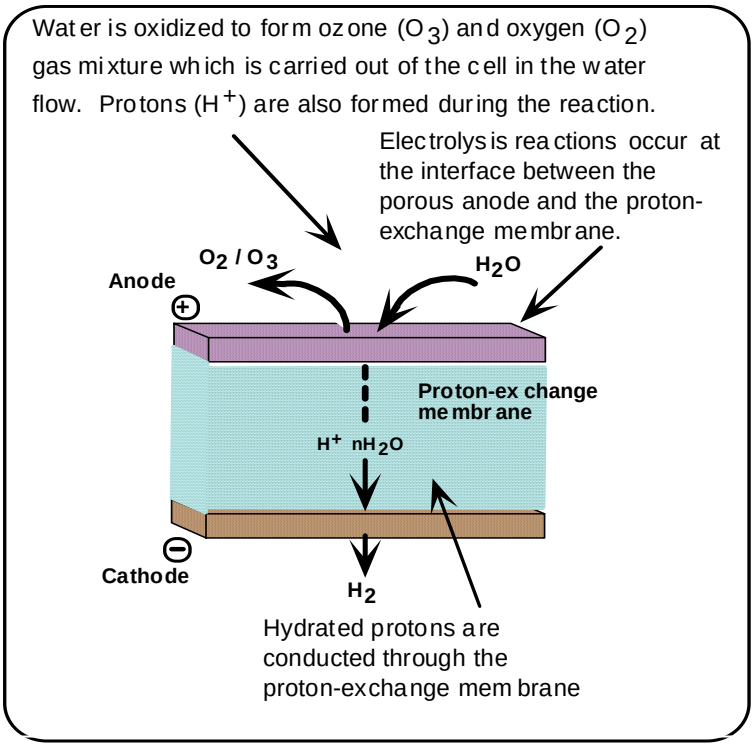

Figure 1- Principle of electrochemical ozone generation

Source: Lynntech Inc.

recombined at the cathode. Electrons are conducted to the cathode via the external electronic circuit.

Sanitizer Treatments

Inoculated cantaloupes obtained after drying for a period of 12 h at room temperature were treated as follows: unwashed (control); washed with tap Water; 200 ppm chlorine dip; 2% lactic acid spray or 30 ppm ozone dip. Inoculated cantaloupes were washed under running tap water. The melons were rotated and the treatment was carried for 3 min. The treatment with chlorine solution was performed by submerging the melons in 6 L of 200 ppm chlorine solution and manually rotating the melons in order to ensure complete coverage and contact of the surface with the solution. The treatment was applied for 5 min. Treatment with 2% lactic acid solution was performed by spraying the surface of the inoculated melon with a Roundup^{TM} herbicide sprayer (The Fountainhead Group INC, NY). The sprayer was adjusted in such a way that it delivered 225 ml of the 2% lactic acid solution at 55 °C solution in 15 s and the treatment was carried out for 2 min. Treatment with 30 ppm ozone was performed by submerging the inoculated melons into a plastic tub containing ozonated water. Ozone was generated by the ozone generator with water as its source. The ozone gas generated was pressurized by the internal operation of the cell without a compressor. Deionozed water is pumped from the anode reservoir where the ozone gas liquid separation occurs. The ozone gas output from the gas liquid separator was fed directly into the injection wells of a sterilite (69.1 L) plastic container which was filled with 30 L of deionized water into which the inoculated melons were placed. The deionized water was

continually drawn from one end of the container, passed through an ozone contacting device assembled by Lynntech Product Development, then returned to the opposite end of the container. The concentration of ozone in the water bath was measured, using a Shimadzu UV-3101PC UV/VIS/NIR scanning spectrophotometer (Shimadzu Scientific Instruments, Inc., Maryland, USA). The concentrations of ozone in the water were taken at three different points: Ozonated water inlet, ozonated water bath center, and water outlet. Fisher-Sci 10 mm Quartz Cuvettes used in sample analysis. Ozone absorbance was recorded at 255.3 nm wavelength. The concentration of ozone in water was measured using the formula

Ozone [ppm] in water = absorbance x 160.5

The concentration of ozone was allowed to reach 50 ppm before placing the cantaloupes before each group. A total of 18 cantaloupes were tested with ozone. Six cantaloupes were placed in the container in each group. The inoculated melons which were placed in this ozone rich water were manually rotated to ensure even treatment for about 5 min. The actual process of treatment was depicted in Figure 2.

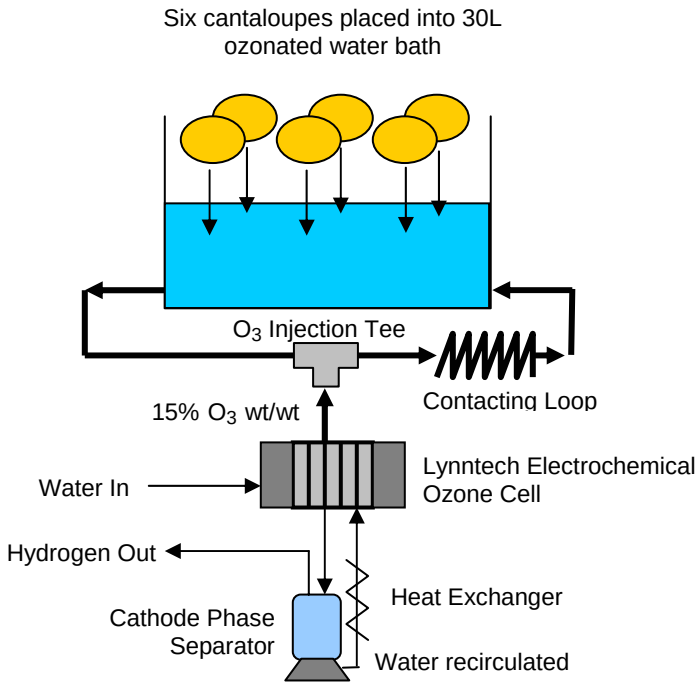

Figure 2- The schematic representation of the entire process in the ozonation of cantaloupes.

Source: Lynntech Inc

Reduction of Bacteria on the Surface of the Cantaloupe by Sanitizing Treatments

To determine the number of surviving bacteria on the surface, core rind samples were collected from each melon using a sterile stainless steel core borer of 10 cm^2 area which was sterilized by dipping in 95% ethanol and flaming. The borer was used to initially cut the surface of the inoculated melon and a sterile scalpel and forceps were used to slice and remove the skin of the cantaloupe of approximately 1 mm - 2 mm deep. Both scalpel and forceps were sterilized between samples by using 95% ethanol and flaming. Three core samples were taken from the rind and one core sample was taken from the stem scar portion of the cantaloupe to determine the difference in the number of bacteria accumulated on the surface and the scar. All the three samples from the rind were composite by placing in a stomacher whirl-pak® bag containing 99 ml of 0.1% peptone water and then pummeled in a stomacher for 1 min at normal speed before examination. The stem scar sample was placed in a separate whirl-pak® bag containing 90 ml of 0.1% peptone water and homogenized for 1 min at normal speed. Viable counts of the rifampicin strain of *S.* Poona were determined by surface plating 1 ml (divided over four plates) and 0.1 ml of the homogenized sample from the whirl-pak® bag and 0.1 ml of the serial ten-fold dilutions from the same homogenized sample onto the surface of previously dried RIF-TSA plates incubating at 37 °C for 18-24 h. The colonies obtained from the surface sampled plates were counted and the final count was divided by 3 in order to get the final count in $\log_{10}$ CFU/10 cm^2.

Transfer of *S.* Poona from the Rind to the Interior Tissue during Cutting

To determine the effect of cutting practices in the transference of pathogens from contaminated rind to the melon flesh, inoculated cantaloupes with or without prior washing treatments, were cut by

Method I Cut: After the corresponding treatment, cantaloupes were cut into two halves using a sterile stainless steel knife. Each half was further cut into and the rind was carefully removed. The interior flesh was cut into cubes and all the cubes were mixed manually with a gloved hand in order to have a homogenous sample. 25 g of the cubes were placed in a whirl-pak® bag along with 225 ml of 0.1% peptone water and pummeled for 1 min at normal speed using a stomacher. Viable counts were obtained by plating 0.1 ml of the homogenized sample onto the RIF-TSA plates followed by incubation at 37 °C for 18-24 h.

Method II Cut: The rind of the inoculated cantaloupes with or without prior washing treatments was first peeled off with a sterile stainless steel knife and then was cut into different sections. The sections were further cut into cubes and the cubes were mixed well manually with a gloved hand in order to get the homogenized sample. 25 g of the cubes were placed in a whirl-pak® bag along with 225 ml of 0.1% peptone water and pummeled for 1 min at normal speed using a stomacher. Viable counts were obtained by plating 0.1 ml of the homogenized sample onto the RIF-TSA plates followed by incubation at 37 °C for 18-24 h

To be able to detect S*almonellae* even if the treatment had resulted in a reduction of the pathogen to levels below the detection limit, melon pieces obtained by the two

cutting methods were qualitatively analyzed for the presence of *Salmonella.* 25 g of the sample was placed in a whirl-pak® bag along with 225 ml of universal pre-enrichment broth (UPB, Difco) and homogenized for 1min at normal speed in a stomacher. The homogenized sample was incubated 37 °C for 24 h. The pre-enriched samples were streaked onto RIF-TSA plates, incubating at 37 °C for 18-24 h. Three colonies from each sample were picked and subjected to biochemical identification to confirm the growth of *Salmonella*.

Survival of *S.* Poona on Fresh-cut Tissue

To determine the growth of bacteria on the fresh cut tissues as affected by cutting method, the untreated (control) and surface sanitized melons were cut by the two methods mentioned above. The melons were cut into cubes with a sterile stainless steel knife based on the method of cut accordingly. The fresh cut tissues were placed in 17 cm x 20 cm Hefty Ziploc (Pactiv Corporation, Lake forest, IL, USA) bags, each bag containing 25 g of the tissue and were stored at 4 °C. The growth of *S.* Poona in the tissue was observed by separating triplicate bags at 0, 3, 6, 9, 12 and 15th days of storage. Three bags from each treatment group were randomly extracted at each sampling day. 25 g of the sample in the ziploc bag was aseptically transferred into a stomacher bag to which 225 ml of 0.1% peptone water was added. The bag was then homogenized for 1 min at normal speed. Viable counts were obtained by plating 0.1 ml of the homogenized sample and serial ten-fold dilutions from the same homogenized sample onto RIF-TSA plates followed by incubation at 37 °C for 18-24 h. Qualitative *Salmonella* analysis was also conducted for each sample following the pre-enrichment method described above.

Confirmation of Isolates

For each day of analysis, typical colonies of rifampicin resistant *Salmonella* colonies were randomly chosen and streaked onto TSA slants and incubated for 24 h. They were then confirmed in triple sugar iron agar (TSIA, Difco)) and lysine iron agar (LIA, Difco) slants. The presence of an alkaline slant (red) and acid butt (yellow) with H_2S production (black) and sometimes with the production of gas in the butt in TSIA confirmed to the presence of *Salmonella* .In LIA, the presence of an alkaline (purple) butt with H_2S production confirmed the presence of *Salmonella.*

Measurement of pH

Surface pH was measured on samples from each treatment group prior to microbiological analysis. A Markson Model 612 portable digital pH meter (Markson Science, Inc., Phoenix, AZ) was used with a flat surface probe (Markson Science, Inc). The pH meter was properly calibrated and sanitized prior to use on each day of analysis. This measurement was conducted in triplicate.

Data Analysis

Six replicates per treatment and three samples per replicate were conducted for each experiment. Means of the samples for each replicate and the means of the replicate data from each experiment were subjected to SAS (Statistical Analysis Systems Institute) for ANOVA (Analysis of Variance) and GLM procedure. The differences between the treatments were analyzed by analyzing the difference within a given treatment over various sampling times. Least square means were determined and standard error/percent difference was used to determine mean differences at $p<0.05$.

RESULTS AND DISCUSSION

Preliminary Studies

Growth curve characteristics

To prevent any interference of the background microflora in the enumeration of the inoculated microorganism, the strain used in this study was marked with resistance to rifampicin. The growth of rifampicin resistant strain of *S.* Poona showed no significant difference ($P<0.05$) when compared with the growth of the parent strain in TSB at 37 °C over a 24 h period (Figure 3). The generation time, was determined by the ratio of the lag phase time and the number of generations. It was found that the rifampicin resistant strain of *S.* Poona had a generation time of 23 min and the parent strain had a generation time of 24 min. The stationary phase for both the strains started after 12 h of incubation at 37 °C in TSB.

Heat tolerance study

The heat tolerance characteristics of both strains at 50 °C and 60 °C are shown in the Figures 4 and 5 respectively. At 50 °C both the rifampicin-resistant strain of *S.* Poona and the parent strain exhibited the same level of resistance. Over a 120 s period, the rifampicin-resistant strain showed a reduction of 2.9 $\log_{10}$ CFU/ml whereas the parent strain showed a reduction of 3.2 $\log_{10}$ CFU/ml. At 60 °C during a 60 s period, the rifampicin-resistant *S.* Poona and the parent strain showed a reduction of 3.1 $\log_{10}$ CFU/ml and 3.7 $\log_{10}$ CFU/ml respectively. From the results obtained, we can conclude that there were no significant differences ($P<0.05$) between the rifampicin resistant strain

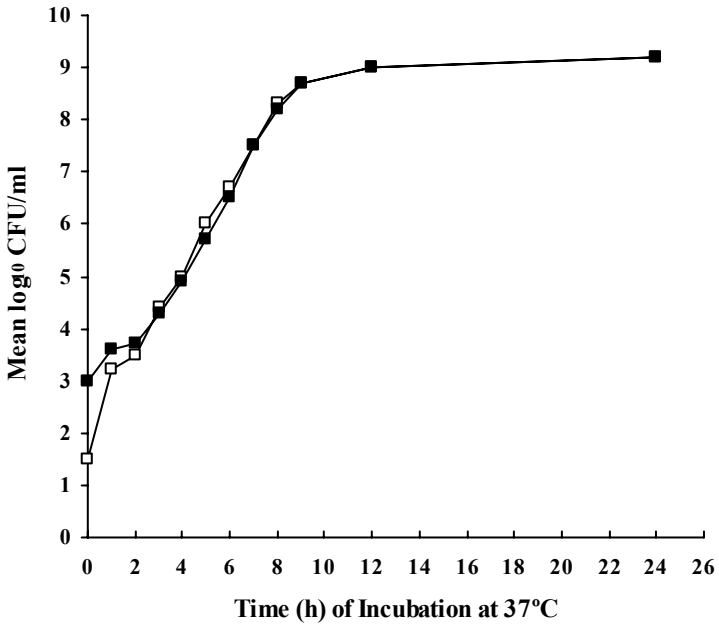

Figure 3: Growth curves of the parent strain of *S.* Poona (□) and its rifampicin resistant derivative (■) when incubated at 37 °C during a 24 h period.

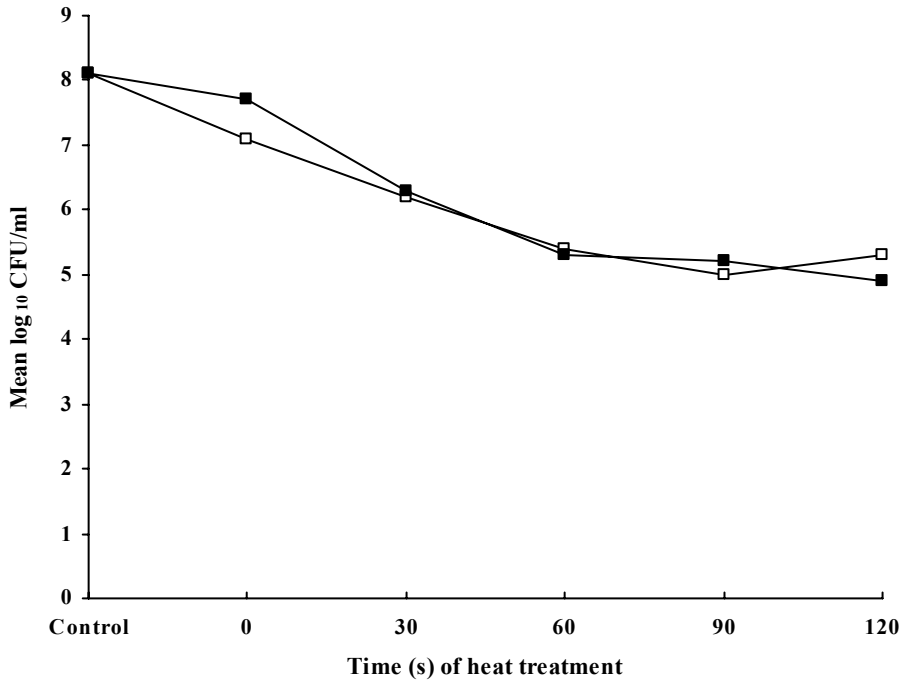

Figure 4: Effect of heat on the survival of *S.* Poona (□) and its rifampicin resistant derivative (■) at 50 °C.

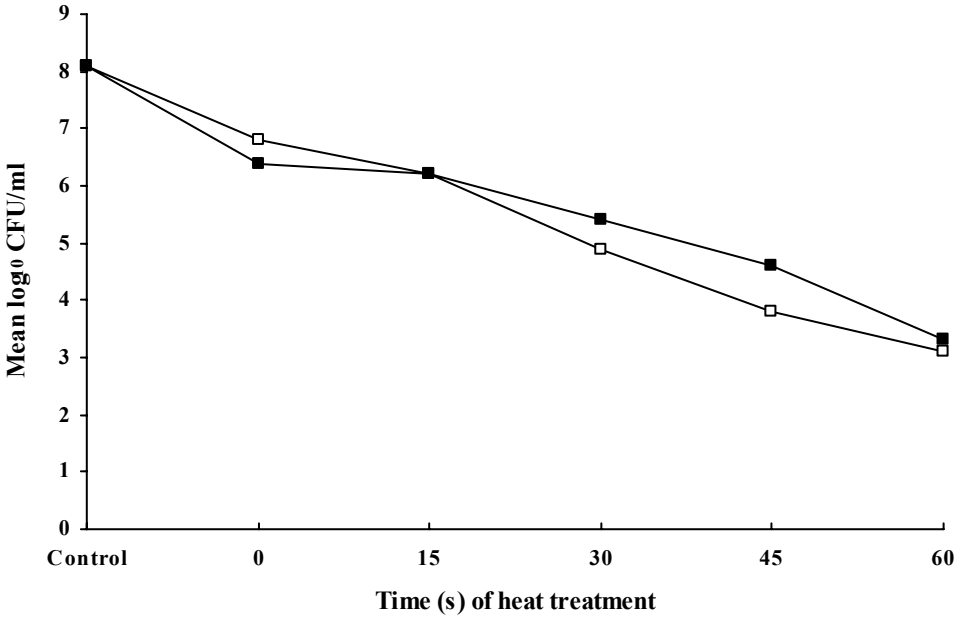

Figure 5: **Effect of heat on the survival of *S.* Poona (□) and its rifampicin resistant derivative (■) at 60 °C.**

of *S.* Poona and the parent strain in their level of resistance to heat either at 50 °C or at 60 °C.

Lactic acid resistance

Resistance patterns of the rifampicin-resistant strain and the parent strain of *S.* Poona to 1% lactic acid are shown in Figure 6. After the first 30 s of contact, the two organisms showed significant differences in resistance to 1% lactic acid when plated on TSA; but no significant differences ($P<0.05$) were found after 120 s period with levels reaching below the detectable limit for both the rifampicin-resistant strain and the parent strain. Sublethally injured cells were enumerated by plating treated samples on TSA + 2% NaCl. The difference in counts between TSA plates and TSA + 2% NaCl was reported as the population of bacterial cells with sublethal injury. It showed that the rifampicin-resistant strain and the parent strain had undergone a stress of 2.1 $\log_{10}$ CFU/ml and 2.4 $\log_{10}$ CFU/ml respectively. This indicates that there was no significant difference ($P<0.05$) in the level of stress undergone by both strains to 1% lactic acid.

Survival of *Salmonella* on the surface of cantaloupes with rotation vs. stationary drying

Recovery of cells from the surface for every 2 hrs of rotation showed no difference in their number of bacteria survived in comparison with the drying of melons for 12 h without rotation. The results are shown in Table 2. The mean value of the log counts obtained from the scar portions of the cantaloupe was found to be 5.8 for both, cantaloupes dried with rotation and cantaloupes subjected to stationary drying The mean counts obtained from the skin of the cantaloupe were found to be 4.5 $\log_{10}$ CFU/ml when rotation was used for drying and 4.6 $\log_{10}$ CFU/ml for non–rotated melons. The above results showed that there were no significant differences between both drying methods.

Reduction of Bacteria on the Surface of the Cantaloupe by Sanitizing Treatments

The initial load of the *S.* Poona in the inoculum used for the study was found to be 7.3 $\log_{10}$ CFU/ml. Data on the populations of *Salmonella* recovered from the skin and scar portions of the cantaloupe after applying different sanitizing treatments are presented in Table 3. The control shows the populations of bacteria recovered from the skin and scar portions of the inoculated cantaloupe after 12 h of drying. These counts were 5.8 $\log_{10}$ CFU/ml and 7.3 $\log_{10}$ CFU/ml respectively. The statistical analysis indicated that these counts were significantly different ($P<0.05$). The same was observed in a study where tomatoes were inoculated with bacteria, the largest numbers of bacteria were recovered from the stem scar, blossom scar and surface scars (Lukasik and others 2001). The counts of the survived bacteria from the skin and scar portions of the cantaloupes after a sole water wash were 6.8 and 7.1 $\log_{10}$ CFU/ml respectively. The higher numbers on the

skin of the water washed cantaloupe compared with those of the control may be due to the cells carried on to the skin from the scar portion during the treatment with tap water and get entrapped in the network without being washed. The cantaloupe having a network of tissues leads to the surface roughness. Complete recovery of the microorganisms from cantaloupes' surfaces is problematic due to surface roughness

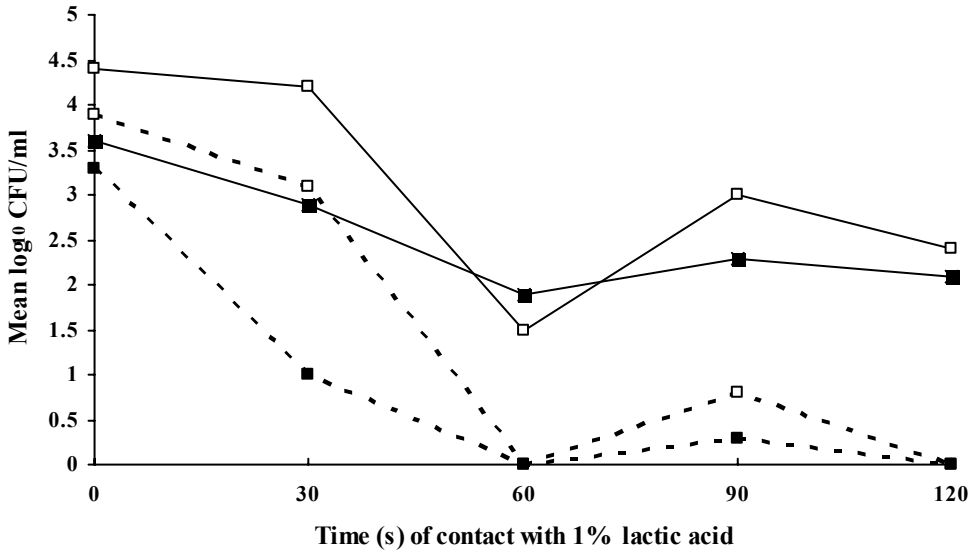

Figure 6: Resistivity and Stress of rifampicin-resistant *S.* Poona (closed squares) and its parent, non-resistant strain (open squares) on TSA (solid lines) and TSA + 2% NaCl (dotted lines) when exposed to 1% lactic acid.

Table 2 – Comparison of the levels of *S.* Poona on different portions of the cantaloupe as affected by drying the melons with and without rotation

Method	Log_{10} CFU/cm^2		
	Skin 1[a]	Skin 2[b]	Stem Scar[c]
Stationary Drying	4.6AX[de]	4.1AX	5.8AY
Drying with Rotation	4.5AX	4.7AX	5.8AY

[a] A piece of 10 cm^2 area cut from the skin near the scar portion of the cantaloupe using a cork borer
[b] A piece of 10 cm^2 area cut from the skin opposite to the scar portion of the cantaloupe using a cork borer
[c] The scar portion of the cantaloupe having an area of 10 cm^2.
[d] The microbial counts expressed are mean values in log_{10} CFU/cm^2.
[e] Means within columns followed by same letter (A, B, C) are not significantly different ($P > 0.05$);
means within rows followed by same letter (X,Y) are not significantly different ($P>0.05$)

(Ukuku and others 2001). This surface roughness provides more surface area for the attachment and this may lead to the entrapments of the cells. Because of this entrapment in the network, it is difficult to retrieve the microorganisms. Numerous reports have suggested that surface structures, including wounds and broken trichomes, as entry sites for pathogens in fruits and vegetables (Getz and others 1983). It was also observed that the attachment of pathogens in lettuce and apples occurs in cracks or cuticles and in broken trichomes, stomata and lenticels (Seo and Frank 1999; Band others 2000; Takeuchi and Frank 2000). It was hypothesized that hydrophobic interactions between the epidermal layer and microbial cells play a major role in facilitating attachment to cuticular cracks, stomata, lenticels and trichomes (Frank 2001). Other authors also have implicated that the population of the cells in the inoculum influences the level of attachment of the microorganisms to apple disks and lettuce leaves (Liao and Sapers 2000; Takeuchi and Frank 2000).

The comparison of *Salmonella* populations after tap water, 200 mg/L chlorine, 2% lactic acid and 30 mg/L ozone is also shown in Table 3. The efficacy of treatments on detachment or inactivation of *Salmonella* on cantaloupe surfaces is dependent on the state and location of the organism on the outer surface and the time of treatment after inoculation (Ukuku and Sapers 2001). Washing with water alone or washing with water followed by washing with 200 mg/L chlorine did not cause a significant ($P<0.05$) reduction of *Salmonella* when compared to the control counts on the rind surface or the scar area. The mean values on the skin and scar portions of the chlorine washed melons were 5.5 and 7.0 $\log_{10}$ CFU/ml respectively. Treatment with 2% lactic acid showed a

significant reduction of *S.* Poona on the skin as well as on the scar portion of the cantaloupe when compared with chlorine treatment or water wash. In comparison with the control, the treatment with 2% lactic acid, resulted in a significant reduction in the counts of *S.* Poona by 2.5 $\log_{10}$ CFU/ml on the skin and 2.7 $\log_{10}$ CFU/ml on the scar regions of the cantaloupe. Results similar to those of lactic acid were obtained by treatment of the inoculated melons with 30 mg/L ozone, which produced log reductions of 2.3 and 2.4 log cycles of *S.* Poona on the skin and scar regions respectively. Treatment with either 2% lactic acid or 30 mg/L ozone exhibited no significant differences in their level of reduction of *Salmonella.* However both the treatments showed significant differences ($P<0.05$) when compared with the chlorine treatment. The presence of organic matter, soil and dirt may lead to the disintegration of chlorine even before it reaches microbial cells, thereby reducing its effectiveness. Even though previous studies indicate the ineffectiveness of chlorine as a good disinfectant for the produce with high organic matter, chlorine was evaluated because it is widely used as a produce sanitizer and could serve as a standard against which to compare the efficacy of other sanitizers. According to the results, < 1 log reduction was obtained using chlorine compared with the control.

Table 3 - Comparison of effects of different sanitizing solutions on the populations of *S.* Poona on the skin and scar portions of the cantaloupe.

Location	Log_{10} CFU/cm^2				
	Control	Tap Water	Chlorine (200 mg/L)	Lactic Acid (2%)	Ozone (30 ppm)
Skin[a]	5.8 ± 0.26AX[cd]	6.6 ± 0.33BX	5.5 ± 0.75AX	3.3 ± 0.45CX	3.5 ± 0.57CX
Stem Scar[b]	7.3 ± 0.30AY	7.1 ± 0.29AX	7.0 ± 0.37AY	4.6 ± 0.46BY	4.9 ± 1.2BY

[a] 3 pieces each of 10 cm^2 area cut from the skin of the cantaloupe using a cork borer
[b] The scar portion of the cantaloupe having an area of 10 cm^2.
[c] The microbial counts expressed are mean values in log_{10} CFU/cm^2 ± Standard Deviation.
[d] Means within rows followed by same letter (A, B, C) are not significantly different ($P > 0.05$);
means within columns followed by same letter (X,Y) are not significantly different ($P > 0.05$).

The results obtained are not uncommon. Studies indicate that the treatment of produce with chlorine at concentrations < 200 mg/L may not be effective. Spray treatment of lettuce with 200 mg/L chlorine was no more effective in removing *E. coli* O157:H7 when compared with water wash (Beuchat 1999). The ineffectiveness of chlorine may be due to different factors, some of them being that the aqueous solution of hypochlorite may not wet the hydrophobic surface of the waxy cuticle and the bio-film present could protect the microorganisms against the lethal effects of the hypochlorite (Adams and others 1989).

Previous studies show that the effectiveness of chlorine as a disinfectant mainly depends on the produce surface (Han and others 2001), on inoculation method (Buchanan and others 1999), on the density of cells in the inoculum (Liao and Sapers 2000; Takeuchi and Frank 2000) and also on the homogenization or blending of the rind. The homogenization of rind in the stomacher may affect the complete recovery of the cells since this may not lead to the release of tightly attached bacteria (Ukuku and Fett 2004). The infiltration of the inoculum into the internal tissue through the stem scar areas may inhibit the efficacy of disinfectants (Buchanan and others 1999; Seo and Frank 1999). The inefficiency of chlorine as a sanitizer may also be due to the change in pH of the wash solution. As the pH of the solution rises, the hypochlorous acid present in the solution splits to hydrogen ions and hypochlorite ions. The hypochlorous acid enters the microorganism and reacts with the –SH group of proteins and ultimately leading to its lysis. These hypochlorite ions formed are not capable of entering the cell and thus has weak disinfectant properties. So when the experiment was conducted at

room temperature in presence of light and air, the hypochlorous acid might have been reduced to chlorite ion thus reducing its activity as a sanitizer, resulting in a poor performance at reducing *S.* Poona on the cantaloupe surface. Studies indicate that the removal of native microflora during washing or sanitizing allow human pathogens to flourish on the produce surfaces (Brackett 1992).

Lactic acid was well-known to be an antimicrobial agent (Bogaert and Naidu 2000). The reductions of the populations due to lactic acid may be attributed to the decrease in pH of the cantaloupe to a level where *Salmonella* cannot survive. Previous studies showed that treatment of tomatoes using acid solutions reduced the survival of pathogenic bacteria (Yoon and others 2004). Previous studies showed the effectiveness of lactic acid and its use as a disinfectant on beef carcasses (Castillo and others 2001). Lactic acid decontamination was considered an important option to control the microbiological quality of fresh produce. It was reported that rinsing in 1% and 2% lactic acid and acetic acid respectively reduced total counts of sprouts by 96% (Becker and Hozapfel 1997).

The reductions obtained by the treatment of the inoculated melons using ozone were similar to those obtained with 2% lactic acid. The antimicrobial action of ozone is due to its strong oxidizing power. Previous studies by Zhao Cranston (1995) indicate that *Salmonella* and *E.coli* populations were reduced by 3 $\log_{10}$ CFU/g in ground pepper after 60 min of treatment with ozonated air. Grapes exposed for 20 min to ozone (8 mg/L) had considerably reduced counts of bacteria, fungi and yeasts (Sarig and others

1996). Kim and Yousef (1998) showed that treating lettuce with bubbling ozone gas decreased the microbial load by 1.5 to 1.9 logs in 5 min.

The populations of *S.* Poona on the skin and scar areas of the inoculated cantaloupe treated with lactic acid and ozone were significantly different ($P<0.05$) when compared with the untreated cantaloupes. They also exhibited significant differences ($P<0.05$) in reducing the populations of *S.* Poona from the skin and the scar areas in comparison with the results obtained by washing with 200 mg/L chlorine.

From the data obtained, it was also hypothesized that the scar portions of the cantaloupe differ significantly in their populations of *Salmonella* from those on the skin. The scar areas of the cantaloupe experienced a greater reduction in populations in all the treatments compared with the skin but there was found to be no difference in their level of reduction statistically ($P<0.05$).

Transfer of *S.* Poona from the Surface to the Internal Tissue during Cutting

The recovery of *S.* Poona from the fresh cut tissue prepared from washed and unwashed melons was analyzed statistically with respect to the methods of cut and is listed in Table 4. In case of the tissue obtained by implementing Method I cut no significant differences were found in the tissues obtained from the melons treated with tap water, 200 mg/L chlorine or 30 mg/L ozone. In case of the tissues obtained by Method II cut from the melons subjected to water wash or 30 mg/L chlorine showed no significant differences. But from the data obtained there was a statistical difference ($P<0.05$) in the rate of transfer of *S.* Poona from the skin to the internal tissue between

the melons washed with 2% lactic acid and the ones treated with tap water, 200 mg/L chlorine or 30 mg/L ozone in both methods of cut.

From the results obtained the method of cut showed a significant difference in the rate of transfer of the organism from the skin to the tissue in all the treatments. Gayler and others (1955) showed that the interior tissue of watermelon could be contaminated if *Salmonella* was present on either the rind of the watermelon or on the knife used for slicing. It was also found that an outbreak associated with melons had resulted from the contamination during the final preparation either through an infected food handler or cross contamination from raw beef to the melon via knives, cutting boards or hands (CDC 1994).

Table 4 - Comparison of effects of different sanitizing solutions on the populations of *S.* Poona in fresh-cut tissue as affected by the method of cut.

	Log_{10} CFU/cm^2				
Cut	Control	Tap Water	Chlorine (200 mg/L)	Lactic Acid (2%)	Ozone (30 ppm)
Method I[a]	2.9AX[cd]	2.7AX	2.9AX	1.2BX	3.4CX
Method II[b]	2.7AX	2.3BY	2.2BY	0.8CY	2.8AX

[a] Tissue obtained from surface sanitized melon by cutting the melon into pieces and removing the rind.
[b] Tissue obtained from surface sanitized cantaloupes by peeling off the rind first and then cutting into pieces.
[c] The microbial counts expressed are mean values in log_{10} CFU/g.
[d] Means within rows followed by same letter (A, B, C) are not significantly different ($P > 0.05$);
means within columns followed by same letter (X,Y) are not significantly different ($P > 0.05$).

Survival of *S.* Poona on Fresh-cut Tissue

Minimally processed fruits and vegetables often provide a good substrate for microbial growth (Nguyen-the and Carlin 1994). Readily available nutrients may allow proliferation of human pathogenic organisms such as *S.* Poona on fresh cut melon. The counts of *S.* Poona recovered from the fresh cut pieces prepared by applying both methods of cut from the washed and unwashed melons after 15 days of storage at 4 °C are shown in Figure 7. During the first 6 days of storage, counts of *S.* Poona remained at similar levels in the melon pieces obtained from cantaloupes that were washed with tap water, 200 mg/L chlorine or 30 mg/L ozone, whereas the *Salmonella* counts on pieces obtained from melons that were treated with 2% lactic acid decreased significantly ($P < 0.05$) after day 3 of storage in comparison with day 0 (results of statistical analysis not shown). On day 9 the populations of *S.* Poona in tissues prepared from lactic acid treated melons decreased to levels below the detection limit of the counting method. In contrast, the *Salmonella* counts in melon pieces obtained from cantaloupes that were treated with tap water, chlorine or ozone, were still higher and the reduction was still less than 1 $\log_{10}$ CFU/g. By the end of 15 days of storage, the counts of *S.* Poona in the fresh cut tissues obtained from the melons washed with tap water, chlorine were reduced by 2.7 and 2.6 $\log_{10}$ CFU/g respectively, and no significant differences were found in their level of reduction when compared to the control, which showed a decrease in count of 2.5 $\log_{10}$ CFU/ml over the 15 days of storage. In the case of ozone treated melons no significant differences were found in the counts of *Salmonella* from day 0 to day 15.

Similar results were obtained over storage between treatments in the case of the tissue obtained by Method II cut peeling off the rind first and then cutting the tissue into cubes but there were significant differences in the number of bacteria surviving or growing on each day in between the methods of cut and in case of the tissues obtained from the melons washed with chlorine, lactic acid or ozone. These differences were not observed in case of control and tap water treated melons. In the case of the cantaloupes washed with 2% lactic acid, the counts of *S.* Poona in the tissue obtained after peeling off the rind reached levels below the detectable limit with in 3 days of refrigerated storage where as in the case of tissue obtained by cutting and then peeling off the rind, the levels of *Salmonella* reached below the detectable limit by the end of 9 days of refrigerated storage. The results obtained from the pre-enrichment steps indicated the survival of *S.* Poona even though they were below the detectable limit.

The results showed that the bacteria still survived after 15 days of storage even after the treatment of the inoculated melons with tap water, 200 mg/L chlorine or 30 mg/L ozone. In the case of the tissue obtained from the cantaloupes treated with 2% lactic acid, the level of *S.* Poona reached below the detectable limit. *S.* Poona, even though present below the detectable limit, has the potential to grow if the temperature becomes favorable. However refrigerated temperatures cannot be relied upon to prevent the growth of pathogenic microorganisms on produce. Populations of *L. monocytogenes* remained constant or grew on a variety of whole and cut produce stored at refrigerated temperatures (Farber and others 1998). The tissue of the cantaloupe which is rich in fructose, glucose, sucrose and other nutrients might favor significant growth.

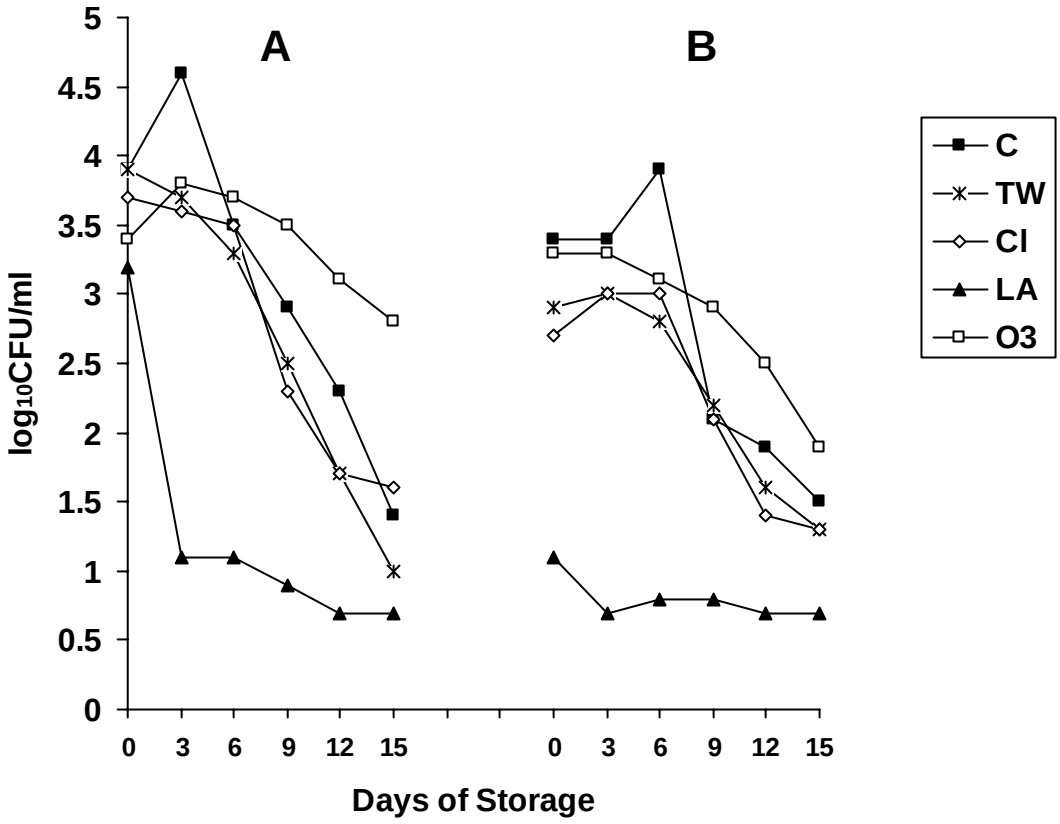

Figure 7 - Survival and growth of rifampicin-resistant *S.* Poona on fresh-cut tissue prepared by (A = Method I cut and B = Method II cut) over refrigerated storage as affected by different sanitizing agents such as Control (C), Tap Water (TW), Chlorine (Cl), Lactic Acid (LA) and ozone (O_3).

The presence of microorganisms even after 15 days of storage in case of tissue obtained from the melons washed with ozone may be due to the rapid decomposition of ozone in water which negates the long term residual antimicrobial activity as seen in storage study with *S.* Poona surviving even after 15 days of storage. Adaptation to stress environments can result in a pathogen becoming better susceptible to survival and growth. *E. coli* O157:H7 (Altekruse and Swerdlow 1996; Buchanan and Edelson 1996) and *Salmonella* (Deng and others 1996; Foster and Hall 1990) for example, are known to adapt to reduced pH and substantially exhibit increased tolerance to stress environments. So, the survival of *S.* Poona even after 15 days of storage may also be due to change in pH on cut tissue. As shown in Table 5, there was a decrease in pH over the storage and, by the end of the 15 days of storage there was a significant difference in the pH of the tissues obtained from the melons washed with 2% lactic acid compared with the values obtained from those of the melons washed with tap water, 200 mg/L chlorine, 30 mg/L ozone or control. This decrease of pH might be due to the fermentation of sugars released during cutting. Adaptation to this low pH along with high moisture content and increased nutrient availability might have favored the survival of *S.* Poona even after 15 days of refrigerated storage. The effect of the pH of fruits and vegetable tissues was reviewed by Lund (1992) who concluded that the pH of most vegetables and of some fruits, would allow the growth of food-borne bacteria. It was shown in previous studies that the low pH of orange juice caused a rapid extinction of *L. monocytogenes* (Parish and Higgins 1989), but *S.* Typhimurium survived and grew in juices of some apple cultivars at pH 3.68 or higher (Goverd and others 1979). The inhibition of *S.* Poona in

the tissue might not only be due to the decrease in intracellular pH but might also be due to the specific effects of undissociated acids on metabolic or other physiological activities (Ita and Hutkins 1991). The decrease in pH over days irrespective of the method of cut might be due to the increase in counts of competing microflora.

After antimicrobial treatments, sub lethally injured food-borne pathogens assume added significance because they are potentially as dangerous as their uninjured counterparts (Kang and Siragusa 1999; Lee and Kang 2001). Various enteric pathogens were observed to multiply on the surface of cut melons (Del Rosario and Beuchat 1995), on shredded lettuce (Abdul-Rouf and others 1993), and on chopped parsley (Wu and others 2000) and under acidic conditions, such as chopped tomatoes (Beuchat and Brackett 1991) and wounded apple tissue (Janisiewicz and others 1999). The tissue of the fresh cut melons is composed mainly of parenchyma cells (Grigorieva and others 1965) containing sugars (Escartin and others 1989; Golden and others 1993), organic acids and other substances that may support the growth of microorganisms. Cantaloupes having a pH of 6.2-6.9 and watermelons with a pH of 5.2-5.7 were recognized as good substrates for growth of *Salmonella* (Wells and Butterfield 1997) and *E. coli* O157:H7 (Dingman 2000). Previous studies indicate the transfer of bacteria from the surface into the tissue during cutting as shown in tomatoes (Lin and Wei 1997), cantaloupes (Ukuku and Sapers 2001).

Table 5 - Mean pH measurements of tissues prepared using both methods of cut from surface sanitized cantaloupes inoculated with *S.* Poona over 15 days of refrigerated storage.

		Storage Time (Days)					
		Mean Values of PH					
Sample Type	Treatment	0	3	6	9	12	15
Method I Cut[a]	Control	5.4AX[cd]	5.2AX	5.3ABX	4.5AX	4.0AY	4.7AY
	Tap Water	6.3BCX	6.0ABYX	5.3ABY	5.3AY	5.7BX	5.8BX
	Chlorine (200 mg/L)	6.0ABX	5.7ABYX	5.0AYZ	5.0AYZ	5.0ABYZ	4.6AZ
	Lactic acid (2%)	5.9ABX	6.3BCX	5.9BCX	4.5AY	4.5AYZ	4.0AZ
	Ozone (30 mg/L)	6.8CX	6.8CX	6.5CX	5.4AY	5.4BY	4.7AZ
Method II Cut[b]	Control	6.3AX	5.7AY	6.0BXY	5.0AZ	4.9AZ	5.3BYZ
	Tap Water	6.0AX	5.5AXY	5.3ACYZ	5.2BZ	5.3AYZ	5.3BYZ
	Chlorine (200 mg/L)	6.4AX	5.7AYX	5.2AY	4.5ACZ	4.8AYZ	4.4AZ
	Lactic acid (2%)	6.3AX	5.4AY	4.5AZ	4.2CZ	4.0BZW	3.5CW
	Ozone (30 mg/L)	6.4AXZ	6.5BX	5.7ABY	5.7BY	6.0CX	5.8BYZ

[a] Tissue obtained from surface sanitized melon by cutting the melon into pieces and removing the rind.

[b] Tissue obtained from surface sanitized melon by peeling the rind first and then cutting the melon to pieces.

[c] All values reported are the mean values of pH from 6 replicates

[d] Paired numbers with in columns expressed by the same letter A or B or C or D are not significantly different.
Paired numbers with in rows expressed by the same letter X or Y or Z are not significantly different.

CONCLUSIONS

The results obtained showed that *Salmonella* Poona attached to the stem scar area of the cantaloupe are more than those on the skin. Sanitizing whole cantaloupe surfaces with either tapwater or chlorine (200 mg/L) was not effective in reducing populations of *Salmonellae* from the surface, having found to cause a reduction of less than 1 log CFU/cm^2. However sanitizing the whole cantaloupe surfaces with lactic acid (2%) or ozone (30 mg/L) appears to be effective in reducing *Salmonella* Poona, resulting in 2.5 and 2.3 log CFU/cm^2 reduction respectively.

Transfer of *Salmonella* Poona from the inoculated rind to the interior flesh during preparation of cut pieces was also demonstrated. Sanitizing whole cantaloupe surfaces with 2% lactic acid was found to greatly reduce this transfer. It was also found that cutting the cantaloupes using a Method II cut was more effective in reducing the transfer of pathogenic bacteria from the surface to the interior tissue when compared with Method I cut.

Once present on fresh-cut pieces, *Salmonella* Poona appears capable of surviving over 15 days of storage at 4°C. The levels of *Salmonella* Poona gradually declined, but survivors were detected after the 15 days of storage in case of the tissue obtained from the melons washed with tap water or chlorine (200 mg/L). For the tissue obtained from the melons washed with lactic acid (2%), the pathogen was not detected after 9 days of storage. Research studies indicate the growth of *Salmonella* under favorable temperatures. This research also confirms previous reports demonstrating that proper refrigeration will control the growth of *Salmonella* (Ukuku and Sapers 2001) and *L.*

monocytogenes (Ukuku and Fett 2002) on fresh cut cantaloupes. The levels of *Salmonella* did not show a decline even at refrigerated temperatures in the tissues obtained from cantaloupes sanitized with ozone (30 mg/L) over 15 days. This may be due to the greater decomposing nature of ozone. Additional research is required for a better understanding of the effectiveness of ozone over storage days in controlling *Salmonella* in fresh cut tissue.

Thus washing and sanitizing play an important role in reducing the populations on the fresh produce. It is noted clearly that chlorine, at concentrations currently permitted for use by the industry to wash fresh fruits can not be relied upon to eliminate or reduce pathogens. New technologies using sanitizing agents of greater lethality are needed to kill microorganisms. Favorable results are obtained with lactic acid (2%) and ozone (30 ppm). However further studies are required to determine the effectiveness of these agents to eliminate microorganisms at higher concentrations without effecting the quality of the product.

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